REMARKS

Reconsideration of the rejections set forth in the Office action mailed September 23, 2003 is respectfully requested. Claims 1-17 and 26-28 are currently under examination; claims 18-25 have been withdrawn.

I. The Invention

For the general purpose of clarification, the applicants refer the Examiner to the illustration of an embodiment of the invention shown in Figures 2A-B. The following description of this embodiment is taken from the specification on page 12, lines 8-29:

"The method, which is illustrated schematically in Figs. 2A-B, employs DNA probes (202,204) prepared from each sample population (e.g. cDNA or genomic DNA libraries), where each probe is labeled with one of two distinguishable labels (206,208), preferably a fluorescent dye, and contains at a terminus one of two "sample identifier" (SID) sequences (210,212), which are able to hybridize with each other. A corresponding library of target sequence clones (214) is prepared, where each clone is attached to a discrete solid surface, e.g. a collection of microbeads (218) or discrete regions on a solid array. Competitive hybridization of the probes to the microbead library is carried out, whereupon probes of the same sequence from two samples hybridize to their complimentary strands on a given region or bead, forming duplexes (220), but with the SID sequences remaining single stranded. *[Note that Figure 2A does not actually show the SID sequences in single stranded form, but depicts them already hybridized either to each other or to a decoder molecule, as described in the following steps:]* The SIDs are then "titrated" by hybridization/ligation (222) of the two types of SIDs from two samples on the same microbead or region. The "remainder" (unhybridized) SID sequences (224) are quantified, preferably via the use of a pair of SID decoder molecules (226,228), which allows the relative abundance of each sequence to be to determined, as the (enhanced) ratio of two fluorescence intensity signals.

As shown in Figs. 2A-B, a 2:1 intensity ratio, which would have been obtained by simply using labeled probes, is enhanced to 3:1. Use of multiply labeled decoders (230, 232) as shown in Fig. 2B, gives even greater enhancement. Flow cytometry analysis can be used to identify and sort DNA clones which are differentially represented in the two samples."

For convenience, the applicants have included a similar drawing showing more of the individual steps described above. In this drawing, probe "B" is present at twice the amount of probe "A". Consequently, after competitive hybridization (bottom left side figure) and "titration" of the SID sequences (bottom right side figure), there are two "remainder" SID's (circled) for probe B.

II. Amendments

Claims 1, 2, 8, 16, 17 and 26 have been amended for clarity, as described below. Claim 28 is amended to correct a typographical error.

The preambles to claims 1 and 26 have been amended to change "at least two nucleic acid populations" to "first and second nucleic acid populations". Support is found, for example, in the body of both claims, which recite first and second nucleic acid populations, as well as in the specification at, for example, page 12, lines 16-17 ("probes of the same sequence from two samples hybridize to their complementary strands"), page 12, lines 2-3 ("probe sequences from the two sources"), and page 27, lines 21-22 ("the DNA probes...prepared from the two different sample sources").

The term "respective populations" has been amended to "first and second populations, respectively" at the 12th line of claim 1 and the 14th line of claim 26. In the same clause, "abundance" has been amended to "relative abundance" for additional clarification.

In claim 26, again in the same clause, the phrase "proportional to the abundance of said sequence" has been amended to "proportional to the relative abundance of the complement of said same sequence". In the probe generation methods described in the specification (as described, for example, in the specification at page 29, lines 20-24 and page 30, line 28 to page 32, line 13), the probes as prepared are complementary to the source nucleic acid sequences, hence the use of the term "the complement of".

In claim 1, the clause "such that said probes are present in duplexes in relative amounts proportional to the abundance of the nucleic acid sequence in the respective populations" has been replaced with "such that the ratio of said first and second probes forming duplexes with said selected sequence is proportional to the ratio of the amount of the selected sequence in the first nucleic acid population to the amount of the selected sequence in the second nucleic

acid population". Similarly, in claim 26, the clause "such that said probes of a given sequence are present in duplexes in relative amounts proportional to the abundance of the nucleic acid sequence in the respective populations" has been replaced with "such that the ratio of said first and second probes having a given sequence and forming duplexes with a complementary reference sequence is proportional to the ratio of the amount of the complementary sequence in the first nucleic acid population to the amount of the complementary sequence in the second nucleic acid population". Support is found in the specification at, for example, page 26, lines 6-10.

In claims 2, 8, 16, and 17, for the sake of clarity, the term "population" or "populations" is amended to recite "nucleic acid population[s]" or "first and second nucleic acid populations".

Claim 2 has been further amended to point out that the plurality of first probes includes different-sequence probes, each having the first SID sequence, and the plurality of second probes also includes different-sequence probes, each having the second SID sequence. Support for a plurality of different-sequence probes is found in the description of probe preparation in the specification, which refers to populations of fragments (e.g. page 30, lines 31-32, which refers to "a population of restriction fragments"; or page 29, lines 20-22: "Probes are prepared from the sources of DNA being analyzed...by preparing a similar restriction digest and appending SID tags to the fragments").

Claim 26 has been further amended to replace "spatially distinct solid phase supports" in step (a) with "microparticles", in accordance with step (c) of the claim.

No new matter is added by any of the amendments.

III. Claim Objections

Claim 8 was objected to on the basis that "said decoder labels" should read "said labeled decoder moieties" to correspond with parent claim 5. The claim has been amended accordingly.

Claim 26 was objected to on the basis that "a given sequence" in (a)(i) should read "said given sequence", since "a given sequence" is used twice in the claim.

In this case, the applicants feel that making this change would alter the meaning of the

claim. As an alternative, for clarification, the first instance of "a given sequence" in claim 26 has been amended to "the same sequence".

IV. Rejections under 35 U.S.C. §112, Second Paragraph

Claims 1, 2, 16, 17, 26 and dependent claims 3-25, 27 and 28 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Item 7. The Examiner stated that "the first part of the step (a) appears to indicate that a reference library does not hybridize with a first and a second probes".

The applicants assume that, by "the first part of the step (a)", the Examiner is referring to the portion of step (a) designated by (i) in the claim. This portion of the claim reads (as amended):

> (i) said first and second probes competitively hybridize with said selected sequence in said reference library,
>
> such that the ratio of said first and second probes forming duplexes with said selected sequence is proportional to the ratio of the amount of the selected sequence in the first nucleic acid population to the amount of the selected sequence in the second nucleic acid population,
>
> and said SID sequences are present as single stranded extensions on said duplexes...

It is unclear to the applicants how this language would suggest that the "reference library does not hybridize" with the probes. The phrase "said first and second probes competitively *hybridize* with said selected sequence in said reference library", as well as the recitation of "said first and second probes *forming duplexes* with said selected sequence", quite clearly indicate that such hybridization occurs. (Applicants also note that the reference library "comprises multiple copies" of the selected sequence, and that the "probes are present in relative amounts...", indicating that multiple molecules of each probe and the reference sequence are available for competitive hybridization. This would be clear to one skilled in the

art, especially on reading the specification.)

The last clause in the claim segment above points out that the terminal SID sequences on the probes are "present as single stranded extensions" on the duplexes; that is, they do not hybridize to the reference library sequence. However, this in no way indicates that the probes do not hybridize with the reference library sequences.

Applicants further note that, in the phrase designated (ii), "said first SID sequences on said duplexes and said second SID sequences on said duplexes hybridize with each other", not with the reference library sequences. This is described, for example, on page 12 and illustrated in Figs 2A-B of the specification.

Item 8. The Examiner stated that the phrase "the respective populations" in claim 1 lacks antecedent basis.

Although applicants believe that the term "the respective...populations", in the context of the claim, would be clear to one skilled in the art, the phrase has been amended to "the first and second populations, respectively", to obviate the issue of antecedent basis.

Item 9. The term "said probes" in section (a)(i) of claim 1 has been amended to "said first and second probes".

Item 10. The Examiner states that "the content of the claim does not mention the relationship between 'said first and second probes' and 'the abundance of the nucleic acid sequence'".

The applicants submit that this relationship is set forth in the claim phrase being discussed, that is, "said first and second probes are present in relative amounts proportional to the relative abundance of the nucleic acid sequence in the first and second nucleic acid populations, respectively" (as amended).

This phrase means, stated differently, that the ratio:

amount of *first* probe present
amount of *second* probe present is proportional to the ratio:

abundance of the nucleic acid sequence in the *first* nucleic acid population
abundance of the nucleic acid sequence in the *second* nucleic acid population.

Applicants submit that this would be clear to one skilled in the art who is familiar with preparation of probes from nucleic acid populations, e.g. for differential expression analysis (as described, for example, in the specification at page 29, lines 20-24 and page 30, line 28 to page 32, line 13).

The second phrase objected to, i.e. the clause "said probes are present in duplexes in relative amounts proportional to the abundance of the nucleic acid sequence in the respective populations", is directed to a similar concept. For clarification, the phrase has been changed to "the ratio of said first and second probes forming duplexes with said selected sequence is proportional to the ratio of the amount of the selected sequence in the first nucleic acid population to the amount of the selected sequence in the second nucleic acid population". See, for example, the specification at page 26, lines 3-16, which further describes this concept (emphasis added):

> Hybridization is competitive in that probe DNA strands with identical, or substantially identical, sequences compete to hybridize to the same complementary reference DNA strands. The competitive hybridization conditions are selected so that *the ratio of the two corresponding probe DNA strands forming duplexes with complementary reference DNA strands reflects, and preferably is directly proportional to, the ratio of the amount of that DNA strand in its population to the amount of the competing DNA strands of identical sequence in their respective population*. Thus, if first and second probe DNA strands from different sources, but with identical sequence, are competing for hybridization with a complementary reference DNA strand, and the first probe DNA strand is at a concentration of 1 ng/μl while the second probe DNA strand is at a concentration of 2 ng/μl, then at equilibrium it is expected that one third of the duplexes formed with the reference DNA would include first probe DNA strands and two thirds of the duplexes would include second probe DNA strands.

The claim terminology would be clear to one skilled in the art who is familiar with the concept of competitive hybridization, especially in view of the description above and the illustration of Figs. 2A-B (where, in a simplified schematic, "Probe A", from a first nucleic

acid population, is present in half as many duplexes as "Probe B", from a second nucleic acid population).

Item 11. The Examiner asserts that claim 2 does not further limit claim 1, in part because "claim 1 only requires a first probe from a first nucleic acid population...and claim 2 requires a plurality of probes derived from said first population".

The applicants maintain that claim 1 is indeed broader than claim 2. Generally speaking, adding elements to a claim should narrow the claim. In this case, claim 1 describes competitive hybridization of two probes complementary to the same sequence (but having different SID sequences) with a single reference sequence. Claim 2 is directed to the same process occurring with additional (multiple) reference sequences and multiple, different-sequence sets of first and second probes from the nucleic acid populations.

The Examiner also stated that it was "unclear that a first nucleic acid population in claim 1 is equal to said first population in claim 2..."

Based on the language of the claim, this is clearly what is intended by the applicants. There is no reason that "a plurality of different-sequence probes" (as recited in claim 2) could not be derived from the nucleic acid populations recited in claim 1.

Item 12. In claims 16 and 17, the term "said populations" is amended to recite "said first and second nucleic acid populations".

Item 13. The preamble of claim 26 has been amended to recite "first and second nucleic acid populations", thus providing antecedent basis for the later recitation of "said first population" and "said second population".

Item 14. Claim 26 has been amended to replace "said first and second probes", which the Examiner objected to as lacking antecedent basis, with "probes from said first and second nucleic acid populations".

Item 15. This item is similar to Item 7, above.

The Examiner stated that "the first part of the step (a) appears to indicate that a reference library does not hybridize with two different probes".

The applicants assume that, by "the first part of the step (a)", the Examiner is referring to the portion of step (a) designated by (i) in the claim. This portion of the claim reads (as amended):

> (i) said first and second probes competitively hybridize with complementary sequences in said reference library,
> such that the ratio of said first and second probes having a given sequence and forming duplexes with a complementary reference sequence is proportional to the ratio of the amount of the complementary sequence in the first nucleic acid population to the amount of the complementary sequence in the second nucleic acid population,
> and said SID sequences are present as single stranded extensions on said duplexes...

(The following remarks are essentially similar to those addressing Item 7, above.)

It is unclear to the applicants how this language would suggest that the "reference library does not hybridize" with the probes. The phrase "said first and second probes competitively *hybridize* with complementary sequences in said reference library", as well as the recitation of "said first and second probes...*forming duplexes* with a complementary reference sequence", quite clearly indicate that such hybridization occurs.

The last clause in the claim segment above points out that the terminal SID sequences on the probes are "present as single stranded extensions" on the duplexes; that is, they do not hybridize to the reference library sequence. However, this in no way indicates that the probes do not hybridize with the reference library sequences.

Applicants further note that, in the phrase designated (ii), "said first SID sequences on said duplexes and said second SID sequences on said duplexes hybridize with each other", not with the reference library sequences.

Item 16. This item is similar to Item 8, above.

The Examiner stated that the phrase "the respective populations" in claim 26 lacks antecedent basis.

Although applicants believe that the term "the respective...populations", in the context of the claim, would be clear to one skilled in the art, the phrase has been amended to "the first and second populations, respectively", to obviate the issue of antecedent basis.

Item 17. This item is similar to Item 9, above.

The term "said probes" in section (a)(i) of claim 26 has been amended to "said first and second probes".

Item 18. This item is similar, in part, to Item 10, above.

The phrases objected to in claim 26 are the following (as amended):

> wherein said first and second probes having the same sequence, exclusive of the SID sequence, are present in relative amounts proportional to the relative abundance of the complement of said same sequence in the first and second nucleic acid populations, respectively,
>
> and
>
> such that the ratio of said first and second probes having a given sequence and forming duplexes with a complementary reference sequence is proportional to the ratio of the amount of the complementary sequence in the first nucleic acid population to the amount of the complementary sequence in the second nucleic acid population.

(The following remarks are essentially similar to those addressing Item 10, above.)

The Examiner states that "the content of the claim does not mention the relationship

between "said first and second probes" and "the abundance of said sequence".

It appears to the applicants that this relationship is set forth in the claim phrase being discussed, that is, "said first and second probes having the same sequence, exclusive of the SID sequence, are present in relative amounts proportional to the relative abundance of the complement of said same sequence in the first and second nucleic acid populations, respectively" (as amended).

This phrase means, stated differently, that the ratio:

amount of *first* probe present having same sequence
amount of *second* probe present having same sequence is proportional to the ratio:

abundance of the complement of said same sequence in the *first* nucleic acid population
abundance of the complement of said same sequence in the *second* nucleic acid population.

Applicants submit that this would be clear to one skilled in the art who is familiar with preparation of probes from nucleic acid populations, e.g. for differential expression analysis (as described, for example, in the specification at page 29, lines 20-24 and page 30, line 28 to page 32, line 13). (As noted above in section II, the probes as prepared are complementary to the source nucleic acid sequences, hence the use of the term "the complement of".)

The second phrase objected to, i.e. the clause "said probes of a given sequence are present in duplexes in relative amounts proportional to the abundance of the nucleic acid sequence in the respective populations", is directed to a similar concept. For clarification, the phrase has been changed to "such that the ratio of said first and second probes having a given sequence and forming duplexes with a complementary reference sequence is proportional to the ratio of the amount of the complementary sequence in the first nucleic acid population to the amount of the complementary sequence in the second nucleic acid population". See, for example, the specification at page 26, lines 3-16, which further describes this concept (emphasis added):

> Hybridization is competitive in that probe DNA strands with identical, or substantially identical, sequences compete to hybridize to the same complementary reference DNA strands. The competitive hybridization conditions are selected so that *the ratio of the two corresponding probe DNA strands forming duplexes with complementary reference DNA strands reflects, and preferably is directly proportional to, the ratio of the amount of that DNA strand in its population to the amount of the competing DNA strands of identical sequence in their respective population*. Thus, if first and second probe DNA strands from different sources, but with identical sequence, are competing for hybridization with a complementary reference DNA strand, and the first probe DNA strand is at a concentration of 1 ng/μl while the second probe DNA strand is at a concentration of 2 ng/μl, then at equilibrium it is expected that one third of the duplexes formed with the reference DNA would include first probe DNA strands and two thirds of the duplexes would include second probe DNA strands.

The claim terminology would be clear to one skilled in the art who is familiar with the concept of competitive hybridization, especially in view of the description above and the illustration of Figs. 2A-B (where, in a simplified schematic, "Probe A", from a first nucleic acid population, is present in half as many duplexes as "Probe B", from a second nucleic acid population).

In Item 18, the Examiner further stated that it was unclear whether "said sequence" in the phrase "the abundance of said sequence" (original claim) represents "a reference library sequence or a given sequence".

As noted above, the claim has been amended to recite "said first and second probes having the same sequence, exclusive of the SID sequence, are present in relative amounts proportional to the relative abundance of said same sequence...". Accordingly, it is clear that "said same sequence" refers to the "same sequence" which the first and second probes have in common.

The Examiner further stated that it was unclear whether "the SID sequence" represents a first or second SID sequence.

The phrase in question refers to "first and second probes having the same sequence, exclusive of the SID sequence". Since it has already been recited in the claim that each first probe has "...a terminal first sample ID (SID) sequence", and each second probe has "...a

terminal second sample ID (SID) sequence", it would be clear to one skilled in the art that "the SID sequence" in the phrase at question must refer to the first SID sequence in the case of a first probe, and to the second SID sequence in the case of a second probe (since, for example, a first probe would not include a second SID sequence).

Standards of Definiteness

In accordance with case law, the "test for definiteness is whether those skilled in the art would understand the bounds of the claim when read in light of the specification." (*e.g.*, *Miles Laboratories, Inc. v. Shandon Inc.*, 997 F2d 870, 27 USPQ2d 1123 (Fed. Cir. 1993), *cert. denied*, 510 U.S. 1100 (1994); *Orthokinetics, Inc. V. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1 USPQ2d 1081 (Fed. Cir. 1986)). The applicants submit that the subject matter and bounds of the claims would be clear to one skilled in the art, who would be familiar with concepts such as differential expression analysis, preparation of probes from nucleic acid populations, and competitive hybridization.

In view of the foregoing, the applicants submit that the pending claims comply with the requirements of 35 U.S.C. §112, second paragraph.

V. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4403.

Respectfully submitted,

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